ADDENDA FOR ACUTE MANUAL

1. Replace the laboratory water used for culturing and test dilution on p. 9, Section 4, paragraph 4.4.1 to be consistent with the chronic manuals.

2. Replace the analysis of the food on p. 10 in Section 4, paragraph 4.8.3 to be consistent with the chronic manuals.

3. Change the description of holding time on p. 43, paragraph 8.5.4. Does not change holding conditions or limit on holding times, but wording is consistent with chronic manuals.

4. Correct typographical error of how an increase in pH during a toxicity test can be avoided by using a static renewal or flow-through approach.

5. Modify footnotes in two tables of test summary conditions. Listing additional specie that can be used with test condition (In response to comments on proposed rule). Add appropriate reference for name change on p. 61 and 119 for *Notropis leedsi* to *Cyprinella leedsi*. Add this reference to paragraph 6.1.2.

6. For consistency, the wording about where to obtain the Trimmed Spearman-Karber program should be replaced.

7. Fix typographical errors in the footnotes on Table 1 on p. 144 to read Stock #2 instead of Stock #1.

8. Change reference date 1993a to 1994a, 1993b to 1994b, and 1993c to 1993.

SPECIFIC ADDENDA FOR ACUTE MANUAL EPA 600/4-90/027F

1. REPLACE 4.4.1 ON PAGE 9 OF ACUTE MANUALS WITH THE FOLLOWING:

4.4 LABORATORY WATER USED FOR CULTURING AND TEST DILUTION WATER

4.4.1 The quality of water used for test organism culturing and for dilution water used in toxicity tests is extremely important. Water for these two uses should come from the same source. The dilution water used in effluent toxicity tests will depend in part on the objectives of the study and logistical constraints, as discussed in detail in Section 7, Dilution Water. For tests performed to meet NPDES objectives, synthetic, moderately hard water should be used. The dilution water used for internal quality assurance tests with organisms, food, and reference toxicants should be the water routinely used with success in the laboratory. Types of water are discussed in Section 5, Facilities, Equipment and Supplies. Water used for culturing and test dilution should be analyzed for toxic metals and organics at least annually or whenever difficulty is encountered in meeting minimum acceptability criteria for control survival and reproduction or growth. The concentration of the metals Al, As, Cr, Co, Cu, Fe, Pb, Ni, and Zn, expressed as total metal, should not exceed 1 μg/L each, and Cd, Hg, and Ag, expressed as total metal, should be less than 50 ng/L (APHA, 1992). Pesticide concentrations should not exceed USEPA's Ambient Water Quality chronic criteria values where available.

2. REPLACE PARAGRAPH 4.8.3 ON PAGE 10 OF THE ACUTE MANUAL WITH THE FOLLOWING:

4.8.3 New batches of food used in culturing and testing should be analyzed for toxic organics and metals or whenever difficulty is encountered in meeting minimum acceptability criteria for control survival and reproduction or growth. If the concentration of total organochlorine pesticides exceeds 0.15 μg/g wet weight, or the concentration of total organochlorine pesticides plus PCBs exceeds 0.30 μg/g wet weight, or toxic metals (Al, As, Cr, Co, Cu, Pb, Ni, Zn, expressed as total metal) exceed 20 μg/g wet weight, the food should not be used (for analytical methods see AOAC, 1990 and USDA, 1989). For foods (e.g., such as YCT) which are used to culture and test organisms, the quality of food should meet the requirements for the laboratory water used for culturing and test dilution water as described in Section 4.4 above.

3. CHANGE ON PAGE 43 IN REGARD TO HOLDING TIMES FOR EFFLUENT SAMPLES OF ACUTE MANUAL TO THE FOLLOWING FOR CONSISTENCY AND CLARIFICATION:

8.5.4 Sample holding time begins when the last grab sample in a series is taken (i.e., when a series of four grab samples are taken over a 24-h period), or when a 24-h composite sampling period is completed. If the data from the samples are to be acceptable for use in the NPDES Program, the lapsed time (holding time) from sample collection to first use of

the sample in test initiation must not exceed 36 h. EPA believes that 36 h is adequate time to deliver the samples to the laboratories performing the test in most cases. In the isolated cases, where the permittee can document that this delivery time cannot be met, the permitting authority can allow an option for on-site variance for an extension of shipped sample holding time. The request for a variance in sample holding time, directed to the USEPA Regional Administrator under 40 CFR 136.3(e) must include supportive data which show that the toxicity of the effluent sample is not reduced (e.g., because of volatilization and/or sorption of toxics on the sample container surfaces) by extending the holding time beyond more than 36 h. However in no case should more than 72 h elapse between collection and first use of the sample. In static-renewal tests, the original sample may also be used to prepare test solutions for renewal at 24 h and 48 h after test initiation, if stored at 4°C, with minimum head space, as described in SubSection 8.5. Guidance for determining the persistence of the sample is provided in SubSection 8.7.

4. CORRECT TYPOGRAPHICAL ERROR OF HOW AN INCREASE IN PH DURING A TOXICITY TEST CAN BE AVOIDED BY USING A STATIC RENEWAL OR FLOW-THROUGH APPROACH. Redlining shows words that need to be included:

Change paragraph 9.5.9, to read as follows:

9.5.8 Increases in pH may occur in test solutions during acute, static, and non-renewal toxicity tests, resulting in an increase in the toxicity of pollutants such as ammonia. This problem can be reduced by conducting the tests in static-renewal of flow-through mode, rather than a static non-renewal mode.

5. CORRECT THE FOOTNOTE ON THE RECOMMENDED TEST SPECIES

A. Section 5, Table 15: Modify footnote to indicate that specific alternate species from Appendix B, p. 264 can be used with the test condition in Table 15. The footnote should read:

> [1] *Homesimysis costata* (mysid) can be used with the test conditions in this table, except at a temperature of 12°C, instead of 20°C or 25°C, and a salinity of 32-34%, instead of 5-30%, where it is the required test organism in discharge permits.

B. P. 264; Appendix B: Modify footnote to table "Supplemental List of Acute Toxicity Test Species", as follows:

> [1] Test conditions for *Cyprinella leedsi* and *Homesimysis costata* are found in Table 13, p. 61 and Table 15, p. 65, respectively.

C. Addition to references cited: Add the citation for the following reference on p. 27, paragraph 6.1.3 after the beginning of the paragraph as follows:

6.1.3 The test species (AFS, 1991) listed in...

D. Add reference to CITED REFERENCES, p. 119:

AFS. 1991. Common and scientific names of fishes of the United States and Canada. Special Publ. 20, American Fisheries Society, Bethesda, Maryland.

E. Add reference citation in Table: Cite appropriate reference for name change from *Notropis leedsi* to *Cyprinella leedsi*, Table 13, p. 61. Place AFS, 1991 after the species name *leedsi* in the footnote.

6. REPLACE PARAGRAPH ITEM 6 OF 11.2.4.3 ON P. 84 WITH THE FOLLOWING:

6. A computer program which estimates the LC50 and associated 95% confidence interval using the Trimmed-Karber Method, can be obtained through the Environmental Monitoring and Support Laboratory (EMSL), 26 W. Martin Luther King Drive, Cincinnati, OH 45268. The program can be obtained from EMSL- Cincinnati by sending a diskette with a written request to the above address.

7. REPLACE WORDING IN ALL FOOTNOTES ON TABLE 1, P. 144 TABLE ENTITLED "NUTRIENT STOCK SOLUTIONS FOR MAINTAINING ALGAL STOCK CULTURES AND TEST CONTROL CULTURES".

Change the words from Stock #1 in footnotes a, footnote b, footnote c, footnote d, and footnote e to Stock #2.